Electronic Supplementary Material (ESI) for Biomaterials Science.
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Supporting information

Growth Factor Mimic 3,4-dihydroxyphenylalanine encoded bioartificial extracellular matrix like protein promotes wound closure and angiogenesis

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Materials

Expression host *Escherichia coli* tyrosine auxotroph (*E. coli*) *JW2581* was received from Coli Genetic stock center (CT, USA). The CLP synthetic gene in pMK-RQ vector was purchased from Invitrogen (CA, USA) and pQE80-L vector purchased from Qiagen (Valencia, USA). All restriction enzymes and T4 DNA ligase were purchased from New England Biolabs (Ipswich, US). Natural amino acids, M9 salts, 3, 4- dihydroxy-L-phenylalanine (DOPA) and RNA isolation reagent TRI reagent® were purchased from Sigma-Aldrich (Bangalore, India). Isopropyl β- d-1-thiogalactopyranoside (IPTG), ampicillin, Luria–Bertani (LB) broth and imidazole were purchased from Himedia (Mumbai, India). Protein purification His-trap HP column was purchased from GE healthcare (Bangalore, India).

Construction of plasmids and strains

The CLP gene amplified from CLP-pMK-RQ vector with gene specific primers was cloned into pQE80L vector using *BamHI* and *Hind III* restriction enzymes and T4 DNA ligase as per the procedure described by Sambrook and Russel (Sambrook et al., 1989). Then, the ligated CLP-pQE80L vector was transformed into *E.coli* tyrosine auxotroph *(JW2581)*. Expression of CLP protein in tyrosine auxotroph was carried out by growing the bacterial culture in LB broth containing ampicillin (100 µg/mL). After the growth reaches 0.6 OD_{600}, protein expression was induced with 1mM IPTG and incubated overnight at 37 ^{o}C. The expression of the CLP protein was confirmed by running the total cell fraction on a 12% SDS-PAGE.

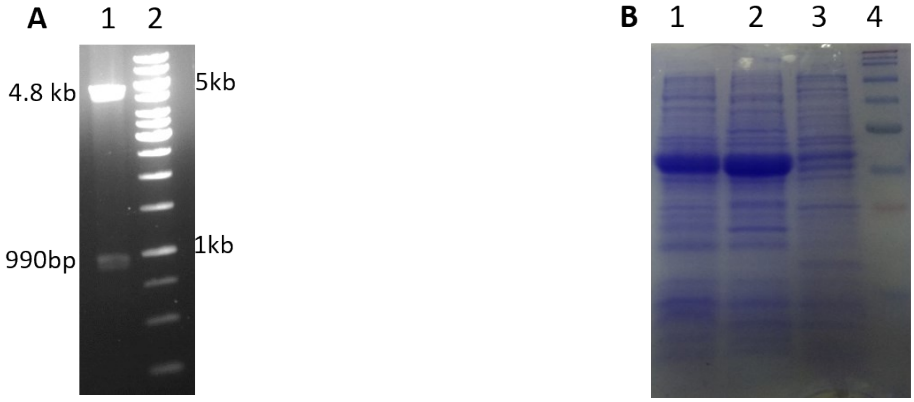

Figure S1. A) Confirmation of CLP gene inserion in pQE80L vector by double digestion of pQE80l+CLP vector by *BamHI* and *HindIII* restriction enzymes, B) CLP and CLPDOPA expression analysis of whole cell lysate lane 1) CLPDOPA, 2) CLP, 3) whole cell lysate without IPTG induction, 4) protein marker.

Confirmation and quantification of orthogonal residue specific DOPA incorporation in CLP

Nitroblue tetrazolium (NBT) staining assay

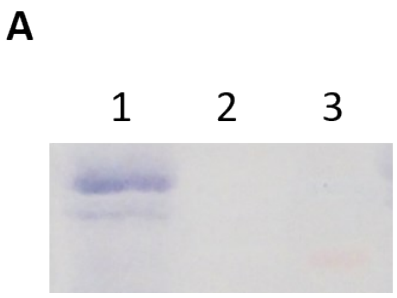

Figure S2. SDS-PAGE resolved Lane 1) CLPDOPA and 2) CLP were trans-blotted into nitrocellulose membrane and the incorporation of L-DOPA in CLPDOPA protein was confirmed by Nitroblue tetrazolium (NBT) staining method.

Matrix Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) analysis

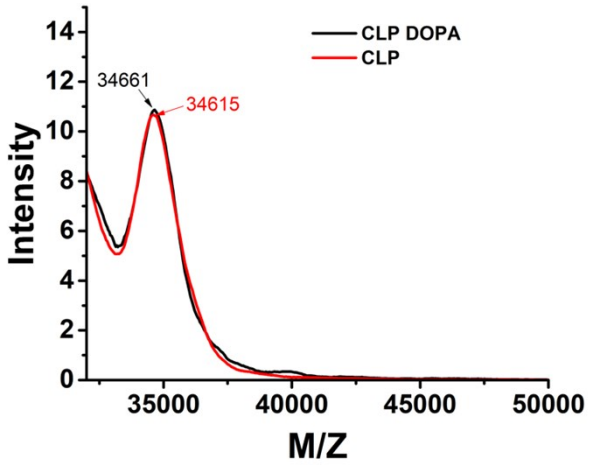

Figure S3. Total mass spectrum of in gel digested CLP and CLPDOPA proteins analyzed by MALDI-TOF .

Table 1: MALDI Mass analysis

Protein	Total number of tyrosine present	Molecular mass of Maldi analysis	% of DOPA incoporation
CLP	3	34615	-
CLPDOPA	-	34661	90%

Amino acid analysis

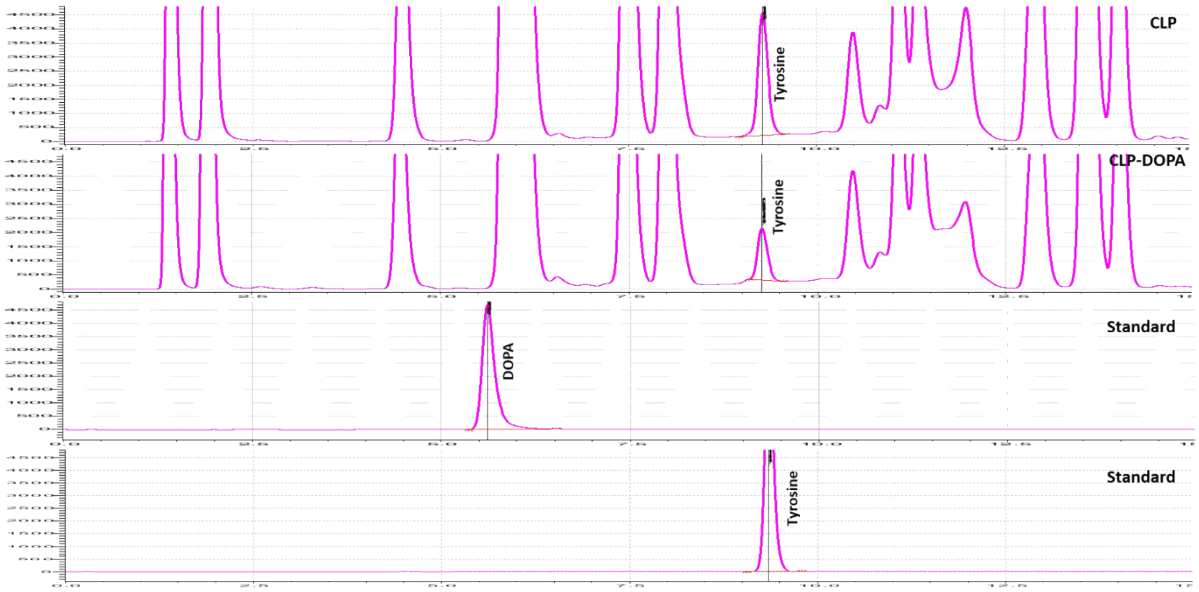

Figure S4. Quantification of orthogonal translational incorporation of L-DOPA into the CLPDOPA protein by HPLC based amino acid analysis.

Table 2. Amino acid analysis quantification of DOPA present in CLPDOPA protein.

Protein	Area of tyrosine peak	% of tyrosine present	% of DOPA incorporation	Number of tyrosine present
CLP	57495387	100	-	3
CLP DOPA	5878331	10	90	-

Purification of CLP and CLPDOPA proteins

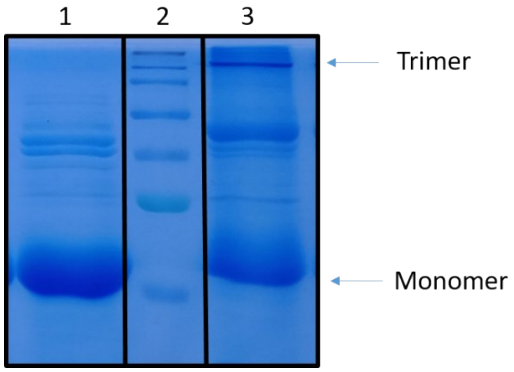

Figure S5. Affinity chromatography purified heat denatured Lane 1) CLP, 2) Marker, 3) CLPDOPA proteins were resolved in SDS-PAGE.

CD secondary structural analysis of CLPDOPA protein

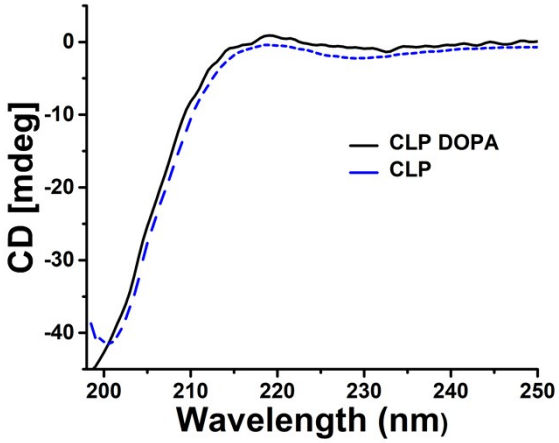

Figure S6. CD spectrometric analysis of triple helical CLPDOPA and CLP.

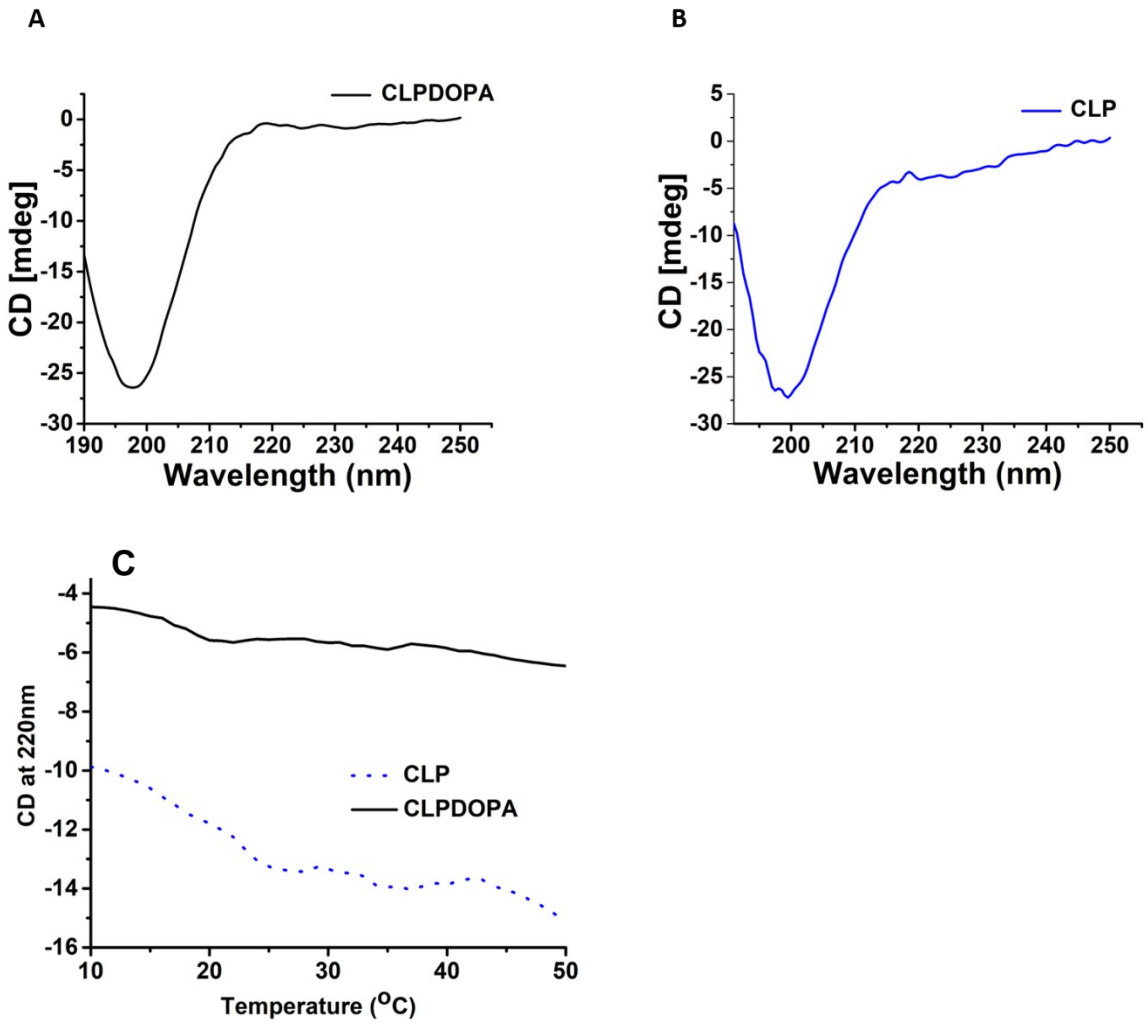

Figure S7. CD spectrometric analysis of renatured A) CLPDOPA and B) CLP. C) CD analysis of refolding of CLP and CLPDOPA at 220nm.

Temperature stability analysis by Fluorescence spectrometry

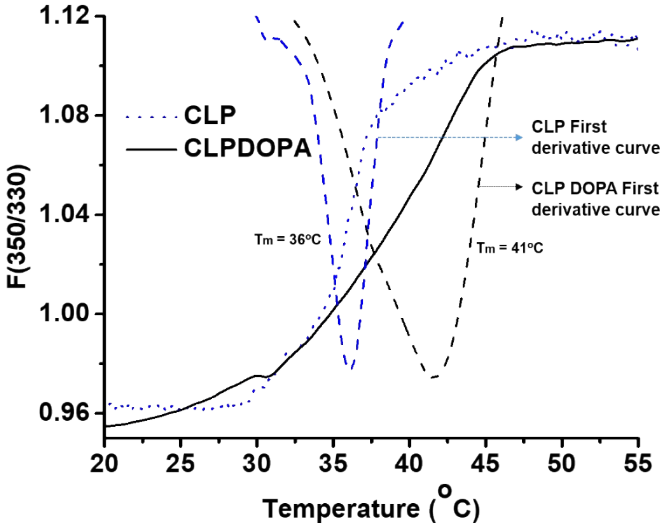

Figure S8. Temperature dependent structural unfolding of CLPDOPA and CLP proteins measured by NanoDSF using intrinsic tryptophan fluorescence emission intensity measured at 350 nm/330 nm.

Self-assembly and fibrillation of CLP and RTT proteins

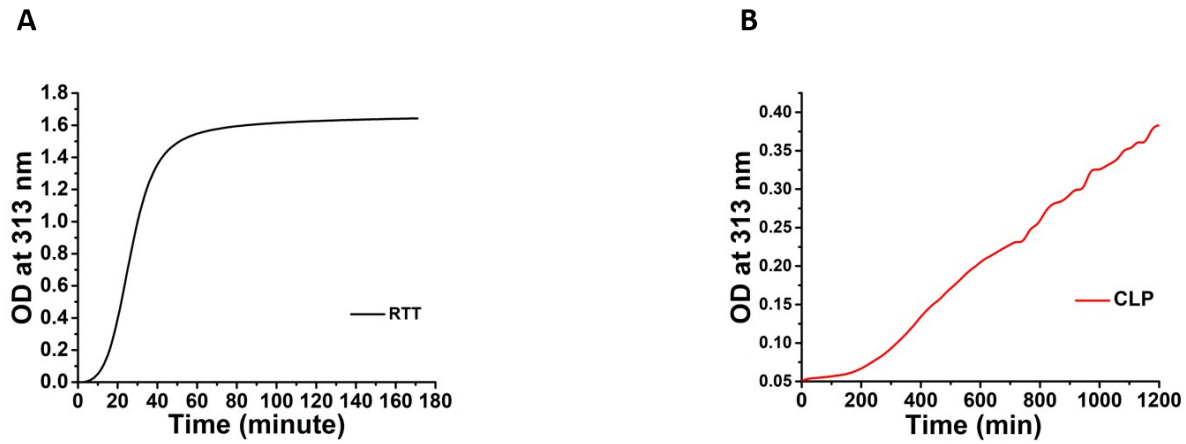

Figure S9. Turbidometric analysis of A) RTT, B) CLP fibrillation kinetics at physiological pH was monitored at 313 nm.

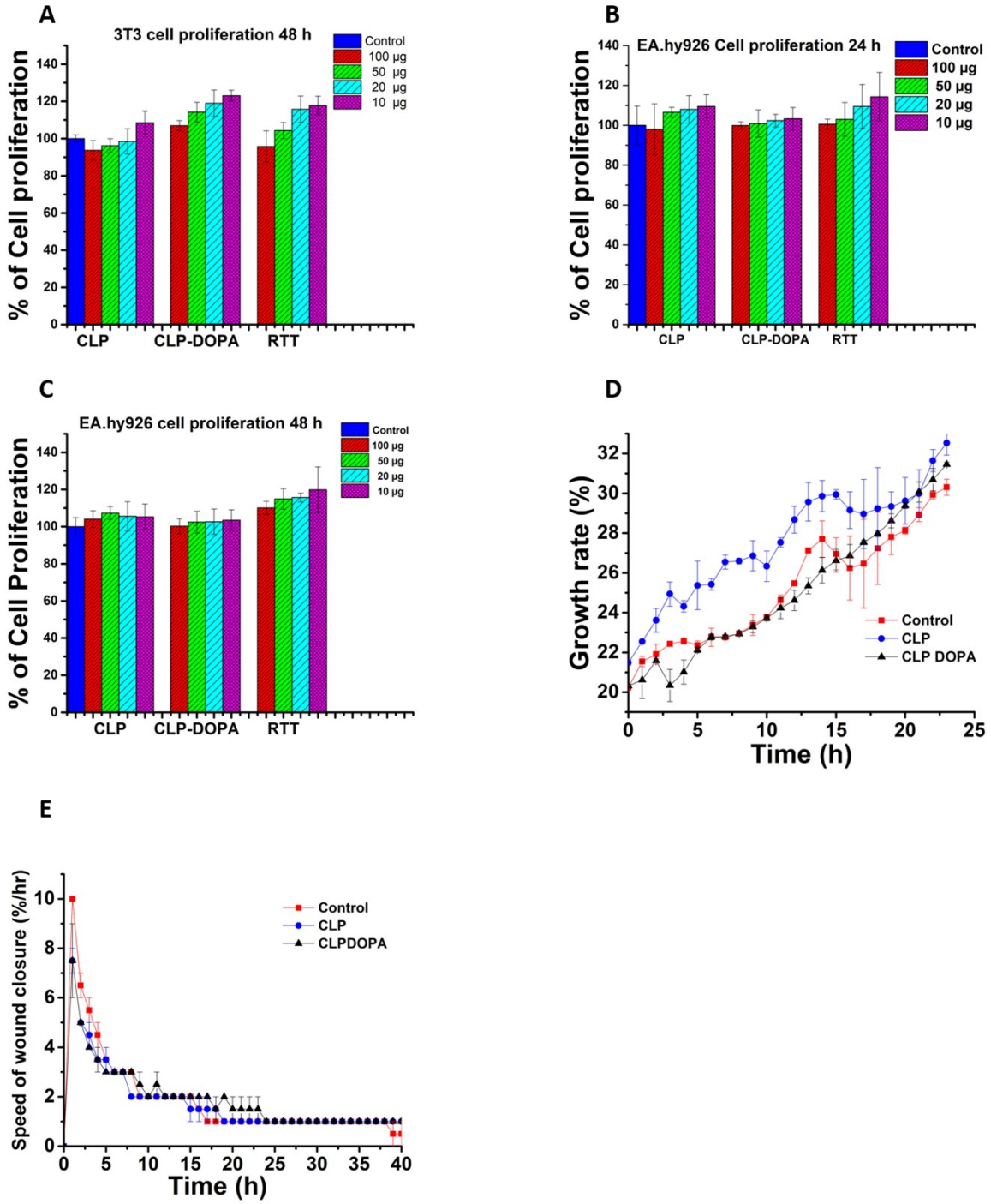

Figure S10. A) 3T3/NIH cell proliferation (MTT assay) at 48 h. B & C) EAhy926 human endothelial cell proliferation (MTT assay) of CLP, CLPDOPA, RTT proteins, D & E) Live cell image monitored EAhy926 human endothelial cell growth rate and speed of wound closure in the presence of 10 µg/mL of CLP and CLPDOPA protein in serum free medium